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FORMATION OF PROPROTEIN AND THERMAL THEORY

POLYCONDENSATION OF FREE AMINO ACID

by

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FORMATION OF PROTOPROTEIN AND THERMAL THEORY  
(POLYCONDENSATION OF FREE AMINO ACID)

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1. Introduction

Lately there has been increasing interest in the origin of biochemical systems and there has also been increasing experimentation on this subject. The object of this type of research is mainly to attempt to convert simple molecules assumed to have existed in the early days of the earth into higher order biochemically significant molecules under environmental conditions similar to those as presumed to have existed in such a geological era. The basis of these studies lies in the fact that life probably evolved on earth ultimately after years of transformation of simple organic and inorganic compounds into more complex organic chemical compounds.

In the present paper, the author would like to summarize our knowledge of the so-called thermal energy theory concerning the origin of amino acids and proteins, particularly concerning the formation of protoprotein.

Many books and articles have been published with respect to the origin of biochemical systems. (1)-(25), (124), (132) Among those well known are Bernal's The Physical Basis of Life (1) Seimei no kigen to seikagaku (Origin of Life and Biochemistry) edited by Fujio Egami, (2) and the third 1957 edition of Oparin's treatise The Origin of Life on the Earth (4) In the same year, there occurred in Moscow, the International Symposium on the Origin of Life on the Earth, the results of which were published by the USSR Academy of Science. (3a) This was later completely translated into English and published as a 650-page treatise by the Pergamon Press in 1959. (3b) Many interesting papers can also be found among those presented at a 1957 Symposium on a New

Concept of Natural Origin, which was co-sponsored by the New York Academy of Science and the American Association for the Advancement of Science.

The composition of the atmosphere in the early days of the earth is important in that it contained the original materials which were to play an important role in ensuing chemical reactions. From astronomical and geological points of view, theories concerning this problem have been proposed by Oparin, (4) Bernal, (1) Rubey, (26) Urey, and Revelle (28). Oparin and Urey have proposed a reducing atmosphere composed of methane, ammonia, hydrogen, and water vapor, while Bernal added carbon dioxide as another important constituent which he assumed to have emanated from the earth's crust. Rubey and Revelle further assumed the existence of some oxidizing agent. Rubey hypothesized an atmosphere of carbon dioxide gas, nitrogen, hydrogen sulfide, and water vapor, and a hydrosphere of carbon dioxide gas, ammonia, hydrogen sulfide, and water, while Revelle assumed an atmosphere of carbon monoxide, carbon dioxide gas, nitrogen, hydrogen sulfide, and water vapor.

It can be imagined that with the escape of hydrogen from the earth's primitive atmosphere and the change to an oxidizing atmosphere, various forms of chemical reactions caused by different forms of energy must have occurred. The energy sources could have been solar radiation, electrical discharge, cosmic rays, radioactive decay radiation, or heat. With respect to the formation of amino acids, using one or more of these different forms of energy, are the following reported studies.

## 2. Formation of Amino Acids

### a. Electrical Discharge

Successful organic synthesis by electrical discharge was first reported by Loebe (29) in 1913, in which case the matter formed has a positive ninhydrin reaction. Miller's research (30)-(34) during 1953 is significant from a new viewpoint, in that he was able to obtain from a Oparin-Urey composition atmosphere, natural as well as synthetic amino acids, fatty acids, oxalic acid, urea, and other compounds. By a similar method, Pavlovskaya (35), Heyns (36), Abelson (37), and Franck (133) were also successful in organic synthesis by electrical discharge.

### b. Ultraviolet Radiation

In a primitive reducing terrestrial atmosphere, it can be assumed that ultraviolet radiation could have been an important source of energy for chemical reactions since no ozone layer would have existed to absorb such radiation. Based on this concept, the

synthesis of amino acids and amine compounds from a mixture of methane, ammonia, and water vapor subjected to various wavelengths of ultraviolet radiation has been reported by Groth<sup>(44)</sup><sup>(45)</sup>, Pavlovskaya<sup>(43)</sup>, Deschreider<sup>(46)</sup><sup>(47)</sup>, Terenin<sup>(42)</sup>, Miller<sup>(33)</sup>, and Ellenbogen<sup>(78)</sup>. Bahadur<sup>(38)</sup>-<sup>(41)</sup> reports on the synthesis of various amino acids using natural sunlight instead of artificial ultraviolet radiation. Matsuda, Senba, and others<sup>(48)</sup>-<sup>(52)</sup> have reported on research involving the decomposition and reformation of amino acids by ultraviolet radiation.

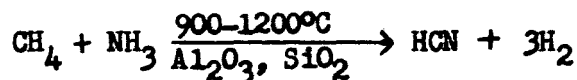
#### c. Radioactivity

Garrison and his collaborators<sup>(53)</sup> and also Hasselstrom and others<sup>(54)</sup> have studied the effect of  $\alpha$  -rays on chemical reactions. Hasselstrom<sup>(55)</sup> has also been successful in synthesizing glycine and aspartic acid from ammonium acetate using  $\beta$  -rays. Paschke and his co-workers<sup>(56)</sup> have detected amino acid formation with  $\gamma$  -ray irradiation, while Vermeil<sup>(58)</sup> and Weeks<sup>(57)</sup> et al report the transformation of amino acids with  $\gamma$  -ray irradiation. Dose<sup>(59)</sup> reports amino acid formation with X-ray irradiation of gas mixtures of methane, ammonia, and other gases.

#### d. Thermal Energy

It can be generally assumed that thermal energy was not an effective agent for decomposing such thermally stable compounds as methane in an environment of moderately low temperatures. On primitive earth however, locally hot spots could easily have existed, such as in the vicinity of volcanic lava flow, and violent chemical reactions could have easily occurred where an atmosphere of methane, ammonia, and other gases came in contact with such hot lava.

Cyanic acid could have been formed in the following manner, with red hot lava acting as a catalyst.<sup>(60)</sup><sup>(61)</sup>



This is a fairly advantageous reaction since it is aided by the presence of sulfur compounds. It is further assumed that this cyanic acid was used for the synthesis of amino acids and fatty acids.

Using ammonia gas, Harada, Johnson, and Fox<sup>(63)</sup><sup>(64)</sup> succeeded in thermally transforming gluconic acid, lactic acid, malic acid,  $\alpha$  -hydroxy-glutaric acid, and other compounds into corresponding amino acids. J. M. Bremar<sup>(65)</sup> and K. Heyns and K. Pavel<sup>(66)</sup> have thermally decomposed amino acids and investigated the formation of amino acids and related compounds. J. Ovo<sup>(67)</sup> and his co-workers have recently obtained various amino acids and other organic acids by heating formaldehyde and hydroxylamine at 80° to 100°C.

As has been described, amino acids as well as other organic compounds can be synthesized by drawing on various sources of energy. It should be noted however that thermal energy as an energy source differs quite drastically from the other four energy sources described. While the latter four are involved in radical chemical reactions, thermal energy is involved mainly with radical chemical reactions only in the high temperature regime, while at relatively low temperatures, that is, at temperatures less than 200°C, ionic chemical reactions are principally expected to occur. It is assumed that the main role of thermal energy at low temperatures was to facilitate as well as to diversify the reaction of unstable intermediate compounds formed by the action of ultraviolet rays and radioactivity. In the next section where the formation of polyamino acids (proteins) from single amino acids will be discussed, the differences in effect between the different forms of energy should become even more apparent.

### 3. Formation of Polypeptides

Today there is considerable research effort being expended by biochemists on the problem of how proteins are synthesized.<sup>(68)(69)</sup> In protein synthesis in living organisms, polypeptides with molecular weights ranging from in the thousands to in the hundreds of thousands are formed by the polycondensation of about eighteen amino acids, and a tremendous variety of proteins are formed by a different sequence of combination of these amino acids. In the laboratory, a number of methods for synthesizing polypeptides have been discovered<sup>(70)(71)(72)</sup>, various naturally occurring peptides have also been synthesized, and many model proteins have been synthesized by methods such as the N-carboxyanhydride method. All of this research work is contributing to a better understanding of the physical, chemical, and biological properties of proteins. On primeval earth, it is generally assumed that organic peptide synthesis could not have occurred, and it can be similarly surmised that organic protein synthesis such as occurs in nature today also could not have occurred. From a comparative biochemical standpoint however, we could assume that the mechanism of primitive protein synthesis has in some way affected the proteins of today and their syntheses.

It is known that 2000 to 4000 cal/mol of energy is required in the reaction which forms one peptide from two amino acids.<sup>(73)</sup> This particular reaction is therefore more in favor of the amino acids, that is in equilibrium, very little dipeptide is formed. On the other hand however, peptide binding energy is known to decrease with increasing peptide size as condensation of amino acids and peptides progresses. In some cases, the reaction is known to be exothermic.<sup>(74)(75)</sup> In general, however, the energy required for the synthesis of peptides from free amino acids can be acquired from sources such as lightening or ultraviolet rays, similar to the case of amino acid synthesis.

**a. Electrical Discharge, Ultraviolet Rays.**

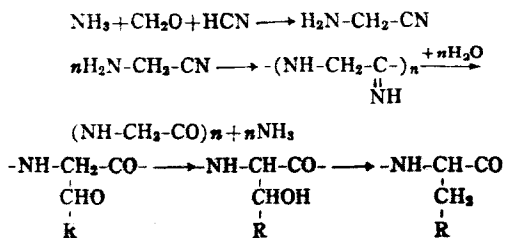
Otozai et al<sup>(76)</sup> have polycondensed glycine and obtained a product insoluble in water, which in turn, upon being hydrolyzed, frees glycine. Bahadur et al<sup>(77)-(79)</sup> have reported peptide formation by exposing an amino acid solution to ultraviolet radiation. These studies are of considerable interest, and it is hoped that more research will be undertaken along these lines.

### **b. Pressure**

Bresler<sup>(80)</sup> reports that gelatine was decomposed by trypsin into peptides, the mixture including the enzyme was then left under several thousand atmospheres of pressure, and a gelatine-like product was obtained. Tongur<sup>(81)</sup> reports on a similar type of experiment. Bresler maintains that under ultra-high pressures, the condensation in peptide formation can occur even for water solution. Since such high pressure reactions are possible near ocean bottoms, this is an interesting point of view. G. Talwar<sup>(82)</sup> on the other hand indicates opposition to Bresler's theory.

c. Polyglycine Hypothesis<sup>(11)</sup>, (83)-(86)

Akabori and his co-workers have demonstrated that the high reaction energy difficulty can be avoided and that by an ingenious method, polyglycine can be synthesized without first synthesizing amino acids, and also that more complex polypeptides can be formed by the attachment of various aldehydes and unsaturated hydrocarbons to the glycine residue as shown in Figure 1.



**Figure 1.**

They also discuss the possibilities of asymmetric polypeptide synthesis making use of the adsorption of polyglycine by asymmetric quartz surfaces. This polyglycine hypothesis came as quite a surprise to workers in this field of research with their customary approach to the problem, and it attracted considerable attention at the 1957 Moscow Symposium on the Origin of Life on the Earth. At present, experimentation is proceeding with the purpose of proving the validity of this hypothesis.

### 3. Thermal Energy

Although since the nineteenth century, a number of attempts have been made to synthesize polypeptides from free amino acids using thermal energy, only a few of these attempts have yielded fruitful results.<sup>(71)(72)</sup> The failures can be generally attributed to the facts that  $\alpha$ -amino acid when heated undergoes decarboxylation, desamination, carbonation, or a diketopiperazine reaction. E. Katschalski<sup>(72)</sup> in a recent 1957 review article states that the method of thermal polycondensation of  $\omega$ -amino fatty acids such as  $H_2N-(CH_2)_nCOOH$ , where  $n$  is 5-6, into polyamides cannot very well be applied to  $\alpha$ -amino acids.

In 1900, Balbiano<sup>(87)</sup> heated glycine in glycerine and obtained a horny substance in addition to diketopiperazine. Curtius<sup>(88)</sup>, Maillard<sup>(89)</sup>, and Abderhalden<sup>(90)</sup> later attempted to repeat this experiment. More recently, Meggy<sup>(93)</sup> has given theoretical consideration to the mechanism of thermal polyglycine formation. Shibata<sup>(91)</sup>, Polyakova<sup>(92)</sup>, Meggy<sup>(93)</sup>, and others have transformed diketopiperazine into polyglycine, and Meggy and Abderhalden have successfully converted glycyglycine into polyglycine. Grimaux<sup>(95)</sup> and Schiff<sup>(96)</sup> report peptide formation by heating aspartic acid.

Kovacs<sup>(97)(98)</sup> has recently demonstrated that the product obtained by heating D-, L-aspartic acid is not a polypeptide but a dehydrated polyanhydroaspartic acid. Vegotsky, Harada, and Fox<sup>(99)</sup> further report that this product is polyanhydroaspartic acid polyhydrate, where a single water molecule is tightly bound to a single anhydroaspartic acid residue. Harada<sup>(100)</sup> has shown that this polymer substance can be formed by the heating of a mono-ammonium salt or a monoamide of fumaric acid, maleic acid, or malic acid, as shown in Figure 2.

In summary, one can say that thermal polycondensation seems to work fairly well for glycine and aspartic acid, but that the same cannot be said for other amino acids.

#### a. Conditions for thermal polycondensation

As can be seen from the literature on the subject, glycine and aspartic acid tend to polycondense easier than the other amino acids. This can be attributed to the fact that the reactant radicals in these amino acids, namely the carboxyl and the amino radicals, occupy the largest fraction of the molecular volume of all of the amino acids, and also to the fact that there is greater stereochemical affinity in the molecular reaction.

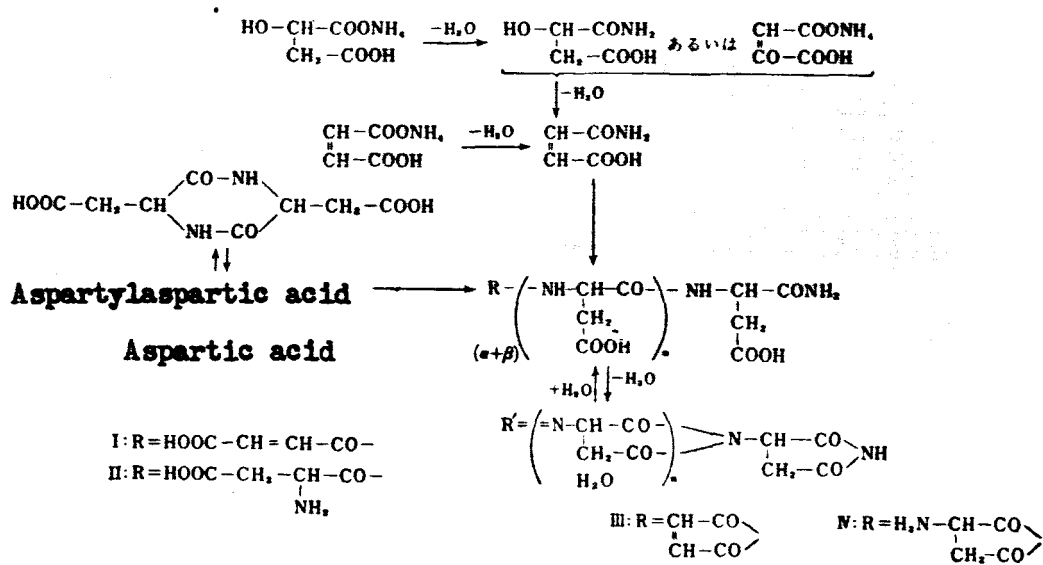


Figure 2. Formation of anhydroaspartic acid hydrate.

From the standpoint of reaction energy, the 2000 to 4000 cal/mol required for formation of the peptide link is not abnormally large compared to normal chemical reaction energies, and we also have to our advantage the fact that the peptide-amino acid binding energy is considerably smaller as already explained.

Another matter of interest here is the fact that since many amino acids are converted to diketopiperazine by heating under certain conditions, there is the possibility that dipeptides might be formed as intermediate products in the reaction converting amino acid to diketopiperazine. Therefore, the problem in synthesizing polypeptides by thermal polycondensation is to discover the reaction conditions which will inhibit diketopiperazine formation but which will facilitate the formation of long peptide chains. It should also be noted that the positive free energy change involved in peptide formation is not the main difficulty in this particular problem.

Past failures in the thermal polycondensation of amino acids can be due to inadequate knowledge of polymers, outdated methods of analysis, as well as possibly an unsuitable choice of reaction conditions. Usually thermal polycondensation of amino acids was performed in the solid state, but sometimes in the liquid phase with glycerine or cresol as solvents. Water was found to be an unsuitable medium for peptide formation, and other solvents were similarly found to be ineffective.



For a thermal polycondensation reaction to proceed properly, it is generally desirable to keep the amount of solvent used to a minimum. Following the initial dipeptide formation, two different types of reactions are permissible, namely one reaction in which a linear polypeptide chain is formed, and the other reaction in which the chain is closed into a ring to form the cyclic compound diketopiperazine. The latter is the primary reaction, and it is assumed to dominate the peptide chain formation reaction at low concentrations. Therefore, the best method for thermal polycondensation of amino acids would be to directly heat and melt amino acids, but this is rendered difficult by the dipolar nature of amino acid molecules. Only in the case of glutamic acid can the dipolarity be eliminated, so that the amino acid can be melted.

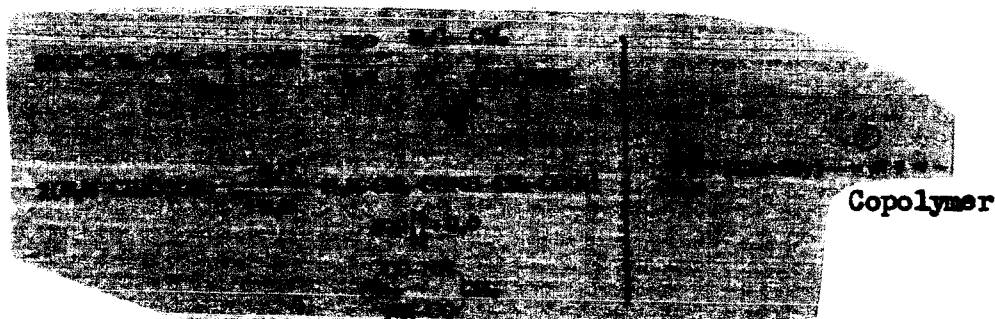
Glutamic acid, upon heating, dehydrates and closes into a ring to form pyrroglutamic acid (5-pyrrolidone 2-carboxylic acid). If for instance glycine is then blended into and dissolved in this liquid pyrroglutamic acid and the solution is allowed to stand for a length of time sufficient for reaction to have occurred, then upon the addition of water and ultrafiltration, a nearly white copolymer of glutamic acid and glycine is obtained.<sup>(101)</sup> This glutamic acid-glycine copolymer has the characteristics given in Table 1.

Table 1. Characteristics of Glutamic Acid-Glycine Copolymer<sup>(101)</sup>

Time heated (hrs)	Yield (after filtration) (grams)	Amino acid composition		Mol weight	N-terminal amino acid	
		Glu (%)	Gly (%)		Glu (%)	Gly (%)
$\frac{1}{2}$	0.12	23	77	12,000	-	-
1	0.39	27	73	11,000	20	80
2	0.51	27	73	18,000	19	81
4	0.71	33	67	18,000	17	83

Remarks: Reaction involved is glutamic acid(0.01 mol) and glycine (0.025 mol) heated at 170°C in CO<sub>2</sub> atmosphere. Molecular weight was determined by N-terminal method. Results differ little from that obtained by light-scattering technique. There seems to be little tendency of ring formation by the N-terminal glutamic acid.

The infra-red absorption spectrum has peaks at the wave numbers 3080, 3300, 1550, and 1650 cm<sup>-1</sup>, quite typical for a polypeptide. The same type of copolymer can be formed by the thermal polycondensation of pyrroglutamic acid and glycylglycine. On the other hand, pyrroglutamic acid and diketopiperazine (both anhydrous) do not form a peptide upon the addition of water but instead are probably in the state of reaction equilibrium shown below.

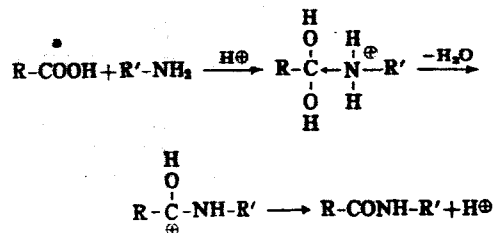


Another example of depolarization of an amino acid by heating and dehydration so that reaction in the molten state can occur is Harada's (102) experiments with lysine. Harada heated and melted free lysine and obtained a homopolymer and various copolymers of lysine by permitting homogeneous reaction to occur with neutral and acidic amino acids.

As an example of forming polymers from amino acid derivatives, which method automatically avoids the dipolar difficulty, a well known example is the formation of polyglycine from glycine ester. (103) More recently, Ovo (104) reports that polyglycine was obtained by heating glycineamide at 80° to 100° C.

The relatively good success achieved with thermal polycondensation of glutamic acid and glycine can probably be attributed to the following factors:

- (1) Easily reacting amino acids are involved;
- (2) Glutamic acid was lactamated (dipolarity was removed), melted, then a second amino acid was dissolved and uniform reaction was allowed to occur.
- (3) Pyrroglutamic acid, the solvent, is also a reactant.
- (4) Pyrroglutamic acid, in addition to being a solvent and a reactant, is also considered to have an oxidation catalytic action in the polycondensation reaction.



The characteristics of pyrroglutamic acid in the above reaction as a solvent, reactant, and catalyst should be an effective guide to later experiments on the thermal polycondensation of amino acids.

b. Formation of multiple component copolymers.

Examples of successful copolymerization are that of a 2-constituent copolymer formed from pyrroglutamic acid and aspartic acid (Figure 3)<sup>(105)</sup> and also 2- and 3-constituent copolymers formed from glutamic acid, aspartic acid, and a third amino acid.<sup>(106)</sup> In both of these cases, the aspartic acid forms polymers in the form of mainly anhydropolyaspartic acid polyhydrate, the same as for the aspartic acid homopolymer as previously described.<sup>(99)(100)</sup>

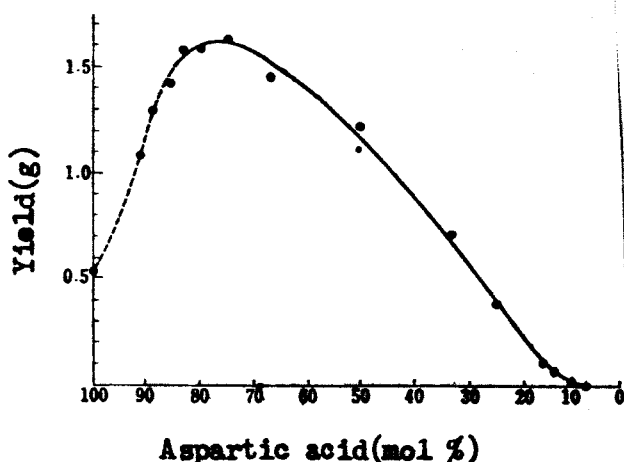
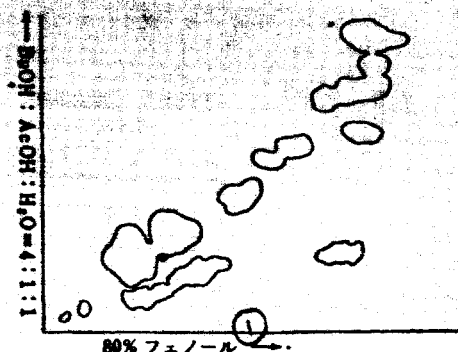


Figure 3. Relationship between yield and ratio of reactants for glutamic acid-aspartic acid copolymer.<sup>(105)</sup> Reactants: (Asp + Glu) = 0.025 mol; reaction conditions, 180°C, 3.5 hours; true line indicates uniform reaction, broken line non-uniform reaction.

The imide form is hydrolyzed in a weak alkali solution and converted into a peptide form. The various studies showed that the presence of an acid amino acid is necessary for the thermal polycondensation of an amino acid, and that neutral and basic amino acids in many cases would polycondense thermally if an acidic amino acid were present.

The knowledge gained with respect to the formation of 2- or 3-component polymers and their properties suggests that the synthesis of copolymers containing all of the amino acids found in proteins is possible. In other words, it should be possible to thermally polycondense the 16 neutral and basic acids in the presence of an excess of glutamic acid and aspartic acid.

Two-dimensional paper chromatography shows that the HCl-hydrolyte of the synthesized protenoid has qualitatively the same composition as the hydrolyte of naturally occurring proteins. (Figure 4)



① Phenol

Figure 4. Two-dimensional paper chromatography of HCL-hydrolylate of protenoid.

Table 2. Composition of 3-component copolymer using ortho-phosphoric acid.<sup>(111)</sup>

Type of copolymer	Amino acid composition		
	Asp	Glu	3rd component
Asp:Glu:Gly	65	11	24
Asp:Glu:Ala	66	5	29
Asp:Glu:Val	72	5	23
Asp:Glu:Leu	67	9	24
Asp:Glu:Ala	70	9	21

Reaction was 160°C, 6 hours in CO<sub>2</sub> atmosphere, 1.5 ml of 80% H<sub>3</sub>PO<sub>4</sub>. Mol ratio of Asp:Glu:3rd amino acid=1:1:1.

c. Effect of phosphoric acid, polyphosphoric acid, and other agents.

In the polycondensation of amino acids where pyrro-glutamic acid was both a reactant and a solvent, other solvents which can also be used include o-phosphoric acid<sup>(109)</sup>, polyphosphoric acid<sup>(110)</sup>, o-phosphorous acid, polysulfonic acid<sup>(111)</sup>, and others, of which the first three are particularly effective. (See Table 2) If we use polyphosphoric acid, which has a large molecular weight, thermal polycondensation, which would normally occur in the 150 to 200°C temperature range, could be made to occur at temperatures below 100°C.<sup>(110)</sup> (See Figure 5) The composition of a multicomponent protenoid formed at 100°C is given in Table 3 as an example. The exact manner in which phosphoric acid or phosphorous acid functions in the thermal polycondensation reaction is not

clear, and at best, we can surmise that these acids contribute to the reaction in the form of N-phosphoryl or some mixed acid dehydrate.

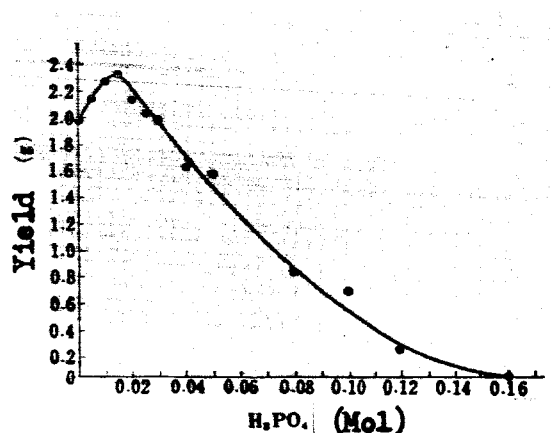


Figure 5. Relation between ortho-phosphoric acid added and yield of glutamic acid-aspartic acid copolymer.<sup>(109)</sup>  
(Asp:Glu = 2:1)

Table 3. Amino acid composition of protenoid\*.<sup>(110)</sup>

Asp	51.1	Ser	0.63	Iso	0.71
Glu	13.0	Pro	1.04	Leu	3.44
Ly	2.79	Gly	2.93	Tyr	3.87
His	2.53	Ala	1.31	Phe	5.87
Arg	1.83	Val	1.33	NH <sub>3</sub>	5.02
Thr	0.55	Met	0.86	不明 (1)	1.16

Note: Tryptophan could not be measured because of HCl-hydrolysis.

\*Synthesized by heating at 100°C for 100 hours with polyphosphoric acid.

Legend: (1) Unknown.

Although sulfuric acid is a good solvent for amino acids and peptides, also a good dehydrating agent, and also probably an acid catalyst similar to phosphoric acid, satisfactory thermal polycondensation could not be attained with sulfuric acid. This is interesting considering the fact that phosphoric acid plays an important role in biochemistry. It has been reported fairly recently that polyphosphoric acid (n = 3 to 10) has been discovered in amoeba, chlorella, yeast, and similar lower order organisms, and that some of these polyphosphates are linked with RNA.<sup>(128)-(130)</sup> Now that is is

known that polyphosphoric acid is effective in the thermal polycondensation of amino acids, then it is quite possible that the polyphosphates which were found linked with RNA in lower organisms might be concerned with the synthesis of proteins.

d. Characteristics of protenoids<sup>(107)(108)(110)</sup>

Protenoids resemble natural proteins in many ways. In addition to containing a large amount of aspartic acid, the amino acid composition of protenoids is reasonable, and the molecular weight of protenoids is in the range 3000 to 9000 which corresponds roughly to protein molecules of  $n_1$  molecular weight. Also with protenoids, various color tests are positive, and infra-red absorption due to anhydroaspartic acid residue as well as the normal peptide absorption are observed. Proténoid is salted in by a dilute salt solution and salted out from a concentrated solution. Ultrafiltration produces a negatively charged band due to the filter paper and electrophoresis, and the substance obtained is fairly homogeneous. In Figure 6 is shown the effect of chymotrypsin and pepsin on a proténoid. It should also be mentioned that, using bacteria, the nutritional value of protenoids has been measured.

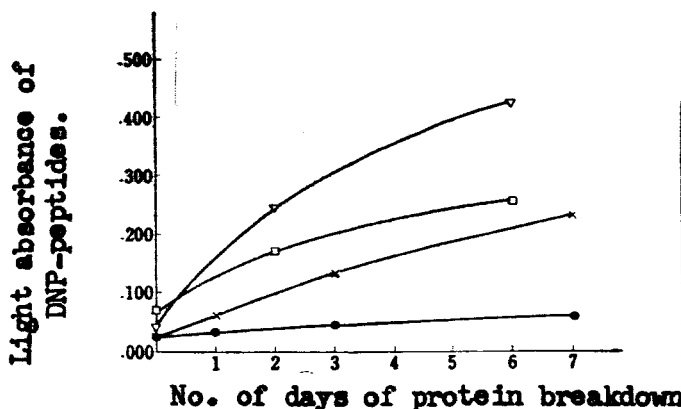


Figure 6. Proténoid decomposition with pepsin and chymotrypsin.

- ▽ Pepsin breakdown of casein.
- Pepsin breakdown of 1:1:1 proténoid.
- α Chymotrypsin breakdown of casein.
- Chymotrypsin breakdown of 2:2:1 proténoid.

e. Proténoid microspherules<sup>(112)</sup> and enzyme-type action.<sup>(113)</sup>

From the viewpoint of the origin of a biochemical system, the proténoid, which has just been discussed, can be considered as a primitive type of protein, and it can be considered quite likely that such proténoids were formed on a primitive earth. The evolution from these proténoids to living cells undoubtedly took an unbelievable length of time involving many cycles of complicated changes, developments, as well as breakdowns. In the course of the evolution of

protenoids, it can be assumed that the formation of a polymer system of proteins, distinct from its environment, would be necessary, and it can also be assumed that enzyme action which would be necessary for a primitive form of metabolic interaction with the environment must exist.

If protenoid is dissolved in a small amount of some salt solution such as brine, then heated and allowed to stand, a white turbidity develops. Under a microscope, this turbidity turns out to be microspherules of 1 to 3 micron size, this size increasing or decreasing readily with the concentration of the salt in the solution, and also with suitable pressure, the microspherules are seen to divide into two and four microspherules similar to normal cell division. Some microspherules are disc-shaped similar to red blood corpuscles. (Figure 7) The formation of protenoid microspherules is interesting not only morphologically, but also from the standpoint that the protenoid microspherules constitute a multimolecular system which is distinct from its environment. The properties of this multimolecular system in a salt solution are quite complicated, and there is still much to be investigated. It has been discovered recently that protenoids have an enzyme type of action, and research is being pursued along this line.



Figure 7. Protenoid microspherules. (112) (Size X1080)

#### f. Characteristics of acidic amino acids.

In his investigation of the amino acid composition of many proteins, Vegotsky<sup>(114)</sup> has discovered that on the average the most abundant amino acid is glutamic acid, followed by aspartic acid and glycine in the order given, and that alanine is the fourth most abundant amino acid. It should be noted that these amino acids thermally polycondense the easiest of all of the amino acids. Allowing our imagination some freedom, one might hypothesize as follows.

Of all of the amino acids, the acidic amino acids are the key elements in the thermal polycondensation process. If one assumes that these acidic amino acids played an important role in the formation of primitive proteins, then the fact that these amino acids are abundant in present day proteins is quite reasonable.

From another viewpoint, if the acidic amino acids were important in the synthesis of primitive proteins, then these same

acidic amino acids must have been of equal biochemical significance after life was organized. To name a few examples, there is the poly-D-glutamic acid found in the cell membrane of bacteria<sup>(115)(116)</sup>, the polyglutamic and polyaspartic acids found in seaweed<sup>(117)</sup>, the pteroylpolyglutamic acid known as a growth factor<sup>(119)</sup>, the phenomenon of transamination, T.C.A.-circuit, the organic synthesis of purines and pyrimidines, and also of proteins.

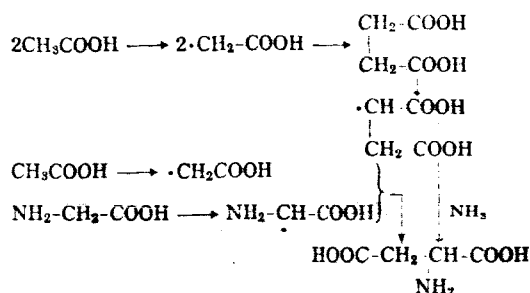
It should be noted that among the activation enzymes necessary for the formation of amino acid-adenosine monophosphate, which constitutes the first step in organic protein synthesis, no activation enzymes for glutamic acid, aspartic acid, or glycine have been discovered<sup>(120)(121)</sup>, so that it is reasonable to assume that these amino acids are activated by some other mechanism.<sup>(122)</sup> This seems to be one of the distinguishing properties of acidic amino acids such as glycine.

Another interesting characteristic of acidic amino acids is that they do not form racemic compounds in the free amino acid state, so that the solubility of the optically active element is smaller than that of racemic compounds. This fact indicates the possibility of optically active matter crystallizing out of a supersaturated water solution of acidic amino acids upon seeding or subjection to shock. Such division cannot be considered entirely impossible on primitive earth, and recently Ogawa and others<sup>(122)</sup> have shown this to be a practical method of dividing glutamic acid.

Concerning the origin of optical activity, a number of theories have been proposed among which are the effect of circularly polarized moonlight or the geomagnetic dipole moment, or quartz adsorption. In the light of present knowledge, another strong possibility which warrants attention is the spontaneous division of glutamic acid under natural conditions, this glutamic acid being a key element in protein synthesis.

Now that we know the characteristics of acidic amino acids as related to primitive protein synthesis, another important question is how the acidic amino acids, glycine, and alanine were formed in the first place. The literature lists ultra-violet radiation, electrical discharge, radioactivity, and other agents as being the sources of energy for amino acid synthesis, and these sources of energy generally tend to favor radical chemical reactions. Among the more common reaction products, one finds acetic acid, glycolic acid, and glycine which contain two carbon atoms, and also alanine propionate with three carbon atoms. Under primitive earth conditions, it seems quite possible that compounds with four or five carbon atoms might be formed by radical chemical reactions. For instance, aspartic acid could be synthesized in the following manner:





Glutamic acid and malic acid are probably synthesized in a manner similar to the formation of aspartic acid. Considering the mechanism just described, it becomes easier to understand why the acidic amino acids came to be produced more abundantly than the other amino acids.

#### g. Criticism of recent thermal energy theory.

Recently Urey and Miller<sup>(124)</sup> published a paper in Science titled "On the synthesis of organic compounds on primitive earth," which was a good overall theoretical treatment of the subject. In this paper, the section describing the thermal energy theory seems to be in considerable error however, and the present author would like to clarify a few of the points concerning this theory.

First of all, the description concerning the thermal stability of amino acids is not altogether correct, since Abelson's research<sup>(125)</sup>, which is quoted, was performed with water solutions and therefore the results would not be applicable to solid amino acids. Also there is no mention of the type of atmosphere in which the thermal stability measurements were made, and the results could differ considerably depending on whether the experiments were conducted in air or in an inert gas medium. The thermal energy theory proposes basically that thermal energy was the efficient energy source in the synthesis of various organic compounds, particular amino acid polymers, and that these polymers formed on primitive earth could have been transported to cooler regions by erupting hot water as well as by other agents.

By means of boiling water in Miller's experiment, gas was circulated, water-soluble matter was collected near the bottom, and various reactions requiring heat were allowed to occur in solution. This author is particularly interested in the parts of Miller's experiments where local high temperature due to electrical discharge and the heat from the boiling water might contribute to the total reaction.

Recently Franck<sup>(113)</sup> published a paper on amino acid synthesis using spark discharge, the paper being titled "thermisch erzeugten Radikalen," which is of interest from the standpoint of the thermal energy theory.

Quoting from E. Katchalski's theoretical paper (1951), Urey and Miller state that peptides were formed according to a known reaction by heating amino acids at 150° to 180°C in the author's laboratory. This statement should be actually interpreted as "according to a reaction of known difficulty" which would then be in accordance with Katchalski's later paper<sup>(72)</sup> of 1958 in which he describes the difficulty of thermal polycondensation of  $\alpha$ -amino acid.

Although Urey and Miller's discussion of energy sources is interesting, they consider solar energy as the only source of thermal energy, they consider the variation in magnitude of this energy, and they also assert that this energy is supplied intermittently. It does seem necessary however to consider what source or sources of energy might be efficient for the synthesis of what types of matter. For instance, in a primitive atmosphere which was assumed to consist of methane, ammonia, water vapor, and other stable compounds, ultraviolet radiation and lightening would probably have been most efficient for the breakdown of these compounds with thermal energy probably playing a secondary role. However in the next step of protenoid synthesis, thermal energy would probably be the more efficient agent as can be surmised from our knowledge of the thermal polycondensation mechanism for polyamino acid synthesis.

As an example of the use of thermal energy for a similar type of synthesis, the reader is referred to a paper by Mora and Wood<sup>(126)</sup> in which is reported the synthesis of polysaccharides with very large molecular weights (5,000 - 30,000) by the thermal polycondensation of glucose. Once large molecules have been synthesized, one can expect ultraviolet rays and radioactivity to be agents of breakdown rather than synthesis of matter.<sup>(131)</sup> In Urey and Miller's paper, it seems that the quantity of energy is well considered in the discussion somewhat to the neglect of the quality of the energy.

Recently Hull<sup>(132)</sup> has reviewed Miller's work from a standpoint of thermodynamics and statistical mechanics and he has shown that ultraviolet rays are more important than heat with respect to the accumulation of the products of synthesis; in other words, such products are decomposed much easier by ultraviolet radiation than by heat. When we consider the fact that volcanoes, which are sources of thermal energy, are localized and intermittent in their activity, the transport of synthesized products to cooler regions, as in Miller's experiment, is within the realm of possibility and such transport is also desirable.

## 5. The Standpoint of Biochemistry of the Past

Near the end of his lecture at the 1906 meeting of the German Chemical Society, Emil Fischer<sup>(127)</sup> said,

"If one could fortunately by chance succeed in producing a real protein by a brutal reaction, for example the co-melting of amino acids in a waterless medium, and if it were even more possible, which is yet unlikely, to identify the synthetic product with a natural substance, then a little would have been attained for albumin chemistry and even less for biology."

Fischer stresses the methodical and sound approach of step-by-step synthesis and analysis as the true organic chemical method for studying protein synthesis. The recent discovery of the structure of the peptide hormone and its complete synthesis is a brilliant example of this organic chemical method. On the other hand, the amino acid melting technique for protenoid synthesis, which Fischer considered to be one of the worst methods, is today after half a century significant in its own right from a different standpoint. This type of chemistry is neither pure organic chemistry nor orthodox biochemistry, and one could probably call this prebiological chemistry. This prebiological chemistry would necessarily have to possess continuity with the biochemistry of living organisms.

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